Pogue-Geile, K.L., Sakakeeny, M.A., Panza, J.L., Sell, S.L., Greenberger, J.S. Cloning and Expression of Unique Murine Macrophage Colony Stimulating Factor Transcripts. Blood, 85:3478 3486, 1995; Goff, J.P., Shields, D.S., Michalopoulos, G.K., Greenberger, J.S. Effects of Hepatocyte Growth Factor and IL-11 on Human Cord Blood CD34+ Progenitor Cells. International Society for Experimental Hematology Meeting, Duesseldorf, Germany, 8/25/95-9/1/95). Current technologies for the growth of stem cells do not address this problem because these technologies are designed to increase the total number of blood cells, not the number of stem cells *per se* (Traycoff, C.M., Kosak, S.T., Grigsby, S., Srour, E.F. Evaluation of Ex Vivo Expansion Potential of Cord Blood and Bone Marrow Hematopoeitic Progenitor Cells Using Cell Tracking and Limiting Dilution Analysis. Blood 85, No. 8:2059-2068 (April 15) 1995; Murray, L., Chen, B., Galy, A., Chen, S., Tushinski, R., Uchida, N., Negrin, R., Tricot, G., Jagannath, S., Vesole, D., Barlogie, B., Hoffman, R., Tsukamoto, A. Enrichment of Human Hematopoeitic Stem Cell Activity in the CD34+Thy-1+Lin- Subpopulation from Mobilized Peripheral Blood. Blood 85, No. 2:368-378 (January 15) 1995). Limiting the differentiation of daughter cells is necessary to grow multiple exact replicas of the original stem cells. By identifying *in situ* the occurrence of cell division and the presence of differentiated cells with microscope imaging, the bioreactor system with z-robot pipette for medium exchange allows solution of this problem: there will be automated exchange of the primary growth medium in a well with a secondary quiescence (*i.e.*, "quieting") medium upon cell division. The first medium promotes proliferation of the original stem cell into exact replicas, and the second medium inhibits differentiation of the resulting daughter cells into committed progenitors.

Understanding and continuing interest in culturing human stem cells obtained from bone marrow and umbilical cord blood has

expanded greatly in the last five years. Human stem cell candidates are identified as CD34+Thy1+Lin- (lin-): they express the cell surface antigens CD34 and Thy1 but not lineage specific antigens (lin-). Antigens are molecules on cell surfaces recognized by specific monoclonal antibodies. CD34+ cells in the bone marrow (approximately 1%) can be isolated by immunomagnetic selection (incubating cells with magnetic beads coated with monoclonal antibodies against CD34 and applying a magnetic field). The subpopulation of CD34+ cells (roughly 1 in 2 to 1 in 4) which do not express antigens associated with differentiated or lineage committed cells can also be removed using appropriate antibodies and immunomagnetic selection or by labeling these antibodies with fluorochromes and flow cytometry. The lin- cells obtained after sorting represent around 1 in 50,000 cells from the original population.

Previous work on developing technology for culturing stem cells has focused on hematopoeitic expansion (*i.e.*, solely increasing the number of committed progeny and mature blood cells) rather than increasing the number of uncommitted lin- cells in the population. For example, Stephen Emerson and Bernhard Palsson (University of Michigan, in collaboration with Aastrom Biosciences, Inc.) developed a batch-operated bioreactor for growing large numbers of CD34+ cells in which culture medium is recirculated over a series of layered individual trays on which stem cells are maintained (Van Zant, Gary, Rummel, Sue A., Koller, Manfred R., Larson, David B., Drubachevsky, Ilana, Palsson, Mahshid and Emerson, Stephen G. Expansion in Bioreactors of Human Progenitor Populations from Cord Blood and Mobilized Peripheral Blood. Blood Cells (1994) 20:482-491). Waste and catabolites are removed continuously from the reactor. Modest increases in numbers of CD34+ cells were detected, but the true lineage specificity of the amplified stem cell was not demonstrated (Van Zant, Gary, Rummel,

Sue A., Koller, Manfred R., Larson, David B., Drubachevsky, Ilana, Palsson, Mahshid and Emerson, Stephen G. Expansion in Bioreactors of Human Progenitor Populations from Cord Blood and Mobilized Peripheral Blood. Blood Cells (1994) 20:482-491).

Based on the results of previous studies in which modest or no increases in the numbers of CD34+ cells were detected (Van Zant, Gary, Rummel, Sue A., Koller, Manfred R., Larson, David B., Drubachevsky, Ilana, Palsson, Mahshid and Emerson, Stephen G. Expansion in Bioreactors of Human Progenitor Populations from Cord Blood and Mobilized Peripheral Blood. Blood Cells (1994) 20:482-491; Verfaille, C.M., Catanzarro, P.M. W. Li. Macrophage Inflammatory Protein 1α, Interleukin 3 and Diffusible Marrow Stromal Factors Maintain Human Hematopoetic Stem Cells for at Least Eight Weeks In Vitro. J. Exp. Med 1994; 179:643-649), the problem of stem cell differentiation during expansion through a combination of biological and engineering technologies was addressed. It was hypothesized that after one cell division one daughter of the two resulting lin- cells might produce inhibitors which limit proliferation and promote differentiation. This hypothesis suggests that the stem cells will be lost if growth conditions are not optimized -- *i.e.*, if the medium is not controlled dynamically to shut down differentiation. This model requires testing with an assay in which individual cell phenotype is identified *in situ*. By detecting the antigens for CD34, Thy1, and Lin with monoclonal antibodies labeled with different fluorochromes fluorescein isothiocyanate (FITC) and phycoerythrein (PE), it was demonstrated that lineage fidelity can be confirmed while maintaining cell viability. These experiments were conducted in single wells of a 96-well plate.

Achieving the goal of maximizing proliferation (*i.e.*, minimizing the time between cell divisions) and minimizing

differentiation of human stem cells clearly requires an automated technology that can significantly reduce the time needed to optimize growth conditions by testing various combinations of the over 30 known molecularly-cloned growth and inhibitory factors. With current tissue culture techniques this task is essentially impossible (Verfaille, C.M. Can Human Hematopoetic Stem Cells Be Cultured Ex Vivo? Stem Cells 1994; 12:466-476).

From a broader perspective, the technology herein will provide a revolutionary means for developing media for tissue culture and protocols for growing cells through the automated testing of a large number of biological variables (*e.g.*, medium composition, environmental conditions, and presence of engineered genes). The opportunity extends into cell biology, molecular biology, the rational development of extracellular matrices for tissue culture and biomaterials, and toxicology. The invention herein will be unique because it enables academic researchers, applied clinicians, or industrial scientists to focus their efforts on understanding the processes of division and differentiation for individual cells. Moreover, the invention herein will be superior to any other available: bioreactors and systems for cell culture which currently are commercially available only allow identification of the properties of populations of large numbers of cells while neglecting phenomena, such as differentiation, which occur at the single-cell level and control the properties of the population.

SUMMARY OF THE INVENTION

The present invention pertains to an apparatus for holding cells. The apparatus comprises a mechanism for incubating cells having a dynamically controlled environment in which the cells are grown, which are maintained in a desired condition and in

which cells can be examined while the environment is dynamically controlled and maintained in the desired condition. The apparatus also comprises a mechanism for determining the state of the cells. The determining mechanism is in communication with the incubating mechanism.

The present invention pertains to a method for holding cells. The method comprises the steps of incubating the cells in a dynamically or controlled environment which is maintained in a desired condition and in which the cells can be examined while the environment is dynamically controlled and maintained in the desired condition. Additionally, there is the step of determining the state of the cells.

BRIEF DESCRIPTION OF THE DRAWINGS

In the accompanying drawings, the preferred embodiment of the invention and preferred methods of practicing the invention are illustrated in which:

Figure 1a is a schematic representation of components of a first embodiment of the present invention.

Figures 1b, 1c, 1d and 1e are details of the chamber of a first embodiment of the present invention.

Figure 2 is a demonstration of the recognition patterns identified by the microscope software which can detect a cell division.

Figure 3 is a representation of the paths of ten human glioblastoma cells (superimposed to a common origin) over a 12-hour period. Scale bars: 100 μm.